

## **Pollen-Mediated Gene Flow from Genetically Modified Herbicide-Resistant Creeping Bentgrass to Compatible Sentinel and Resident Plants**

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A unique opportunity to evaluate methods to monitor potential pollen-mediated gene flow to compatible recipients was afforded by the initial flowering in the summer of 2003 of approximately 162 hectares of experimental fields of creeping bentgrass (*Agrostis stolonifera* L.) genetically modified (GM) for resistance to Roundup® herbicide. In greenhouse tests, seedlings germinated from seeds collected from temporarily deployed sentinel plants of *A. stolonifera* and resident plants of *A. stolonifera* and *A. gigantea* were evaluated for evidence of hybridization based on the presence of a marker gene (CP4 EPSPS). A sampling grid to study the spatial pattern of gene flow in surrounding, largely non-agronomic areas was based on assumptions of pollen viability of three hours and prevailing winds of 10 km per hour from the northwest at the anticipated time of pollen shed. Sentinel plants of *A. stolonifera* were placed in all map directions, but primarily to the south and southeast of the control district that contained the GM fields. Sampling grid intervals for the sentinel plants were smaller close to the control district and increased with distance from the perimeter of the control district. Greenhouse methods for detecting gene flow were based on seedling survival after treatment with Roundup® herbicide in a track sprayer and positive TraitChek™ tests for the CP4 EPSPS marker. Confirmatory laboratory tests for the presence of the engineered CP4 EPSPS marker included PCR and DNA sequencing. The observed frequency of hybridization in sentinel plants (2%) was higher than in resident *Agrostis* spp. (0.03–0.04%). Most hybridizations were observed within 2 km in the direction of prevailing winds from the perimeter of the control district; the maximal distances to which hybridizations were observed were 21 km in sentinel plants and 14 km in resident *Agrostis* spp. The methods we describe to study the spatial pattern of gene flow to compatible non-crop plants can contribute to the environmental risk assessment of genetically modified crops by providing estimates of exposure to marker genes from GM crops on a landscape level that may include non-target, non-crop, and crop plants.